Figure 1. Enzymatic generation of DNA insert encoding a p53-specific shRNA

1/ Anneal stem loop

Primer binding site – sal 1 RE – U – p53 - stem – lo
stem - op

2/ Self prime extension (97 bp)

Primer binding site – sal 1 RE – U – p53 - stem – lo
Primer binding site – sal 1 RE – A – p53 - stem - op

3/ UNG and Piperidine Treatment (78 bp)

p53 - stem – lo
Primer binding site – sal 1 RE – A – p53 - stem - op

4/ Anneal Primer

Primer binding site – sal 1 RE p53 - stem – lo
Primer binding site – sal 1 RE – A – p53 - stem - op

5/ Second Strand Synthesis (78 bp) and polishing

Primer binding site – sal1 RE –T– p53 – stem – loop – stem – p53
Primer binding site – sal1 RE –A– p53 - stem – loop – stem – p53

6/ Sal Digestion (65 bp)

TCGAC –T– p53 – stem – loop – stem – p53
G –A– p53 - stem – loop – stem – p53

Figure 2. Enzymatic generation of DNA insert encoding a random shRNA

1/ Anneal stem loop

Primer binding site – sal 1 RE – U – N19 - stem – lo
stem - op

2/ Self prime extension (97 bp)

Primer binding site – sal 1 RE – U – N19 - stem – lo
Primer binding site – sal 1 RE – A – As19 - stem - op

3/ UNG and Piperidine Treatment (78 bp)
N19 - stem – lo
Primer binding site – sal 1 RE – A – As19 - stem - op

4/ Anneal Primer

Primer binding site – sal 1 RE N19 - stem – lo
Primer binding site – sal 1 RE – A – As19 - stem - op

5/ Second Strand Synthesis (78 bp) and polishing

Primer binding site – sal1 RE –T– N19 – stem – loop – stem – As19
Primer binding site – sal1 RE –A– N_c19 - stem – loop – stem – As_c19

6/ Sal Digestion (65 bp)

TCGAC –T– N19 – stem – loop – stem – As19
G –A– N_c19 - stem – loop – stem – As_c19

Figure 3. Suppression of dEGFP-mediated cell fluorescence using a EGFP-specific shRNA expression plasmid

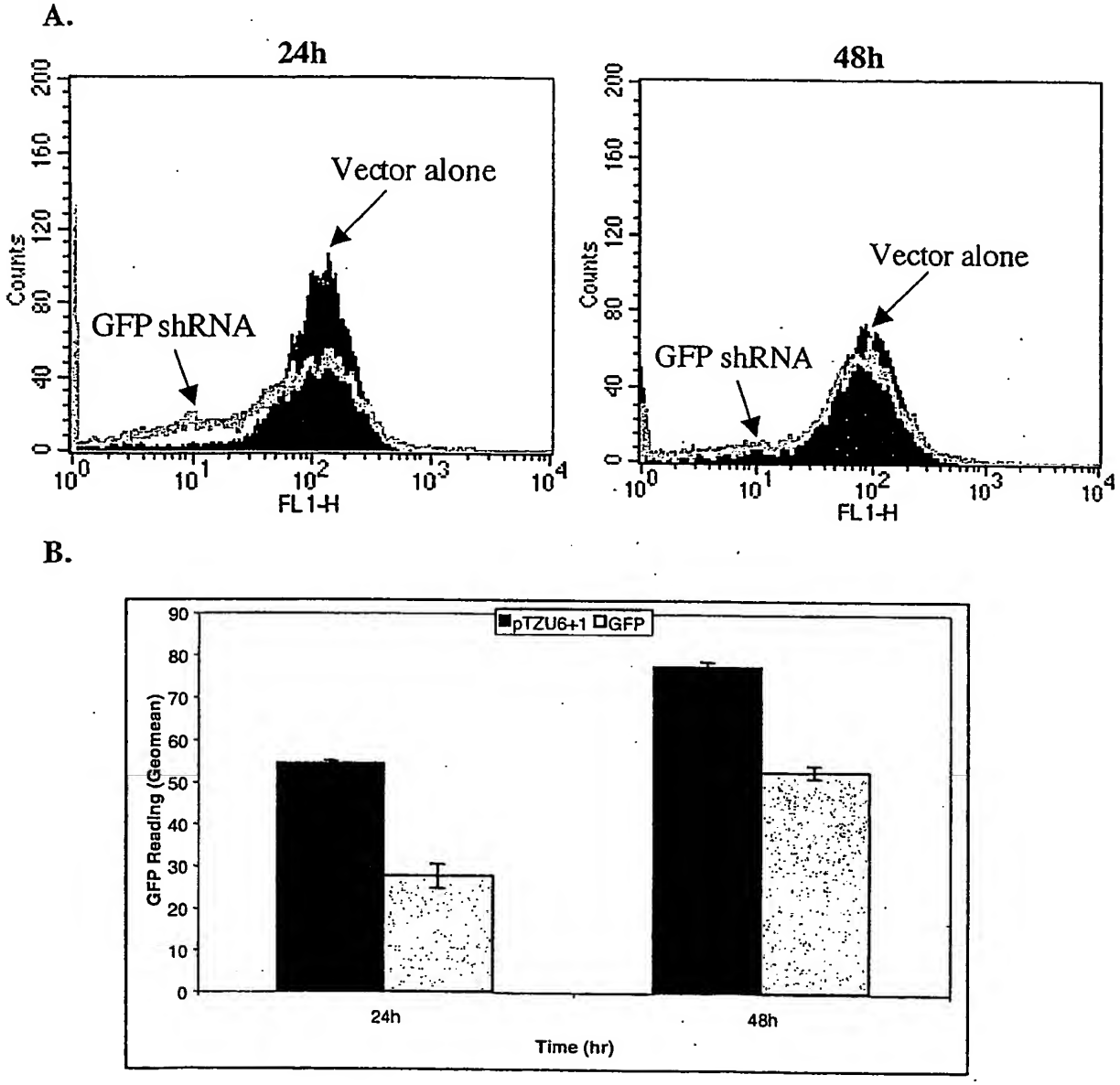


Figure 4. Construction of random shRNA expression library in a modified pLXSN retroviral vector

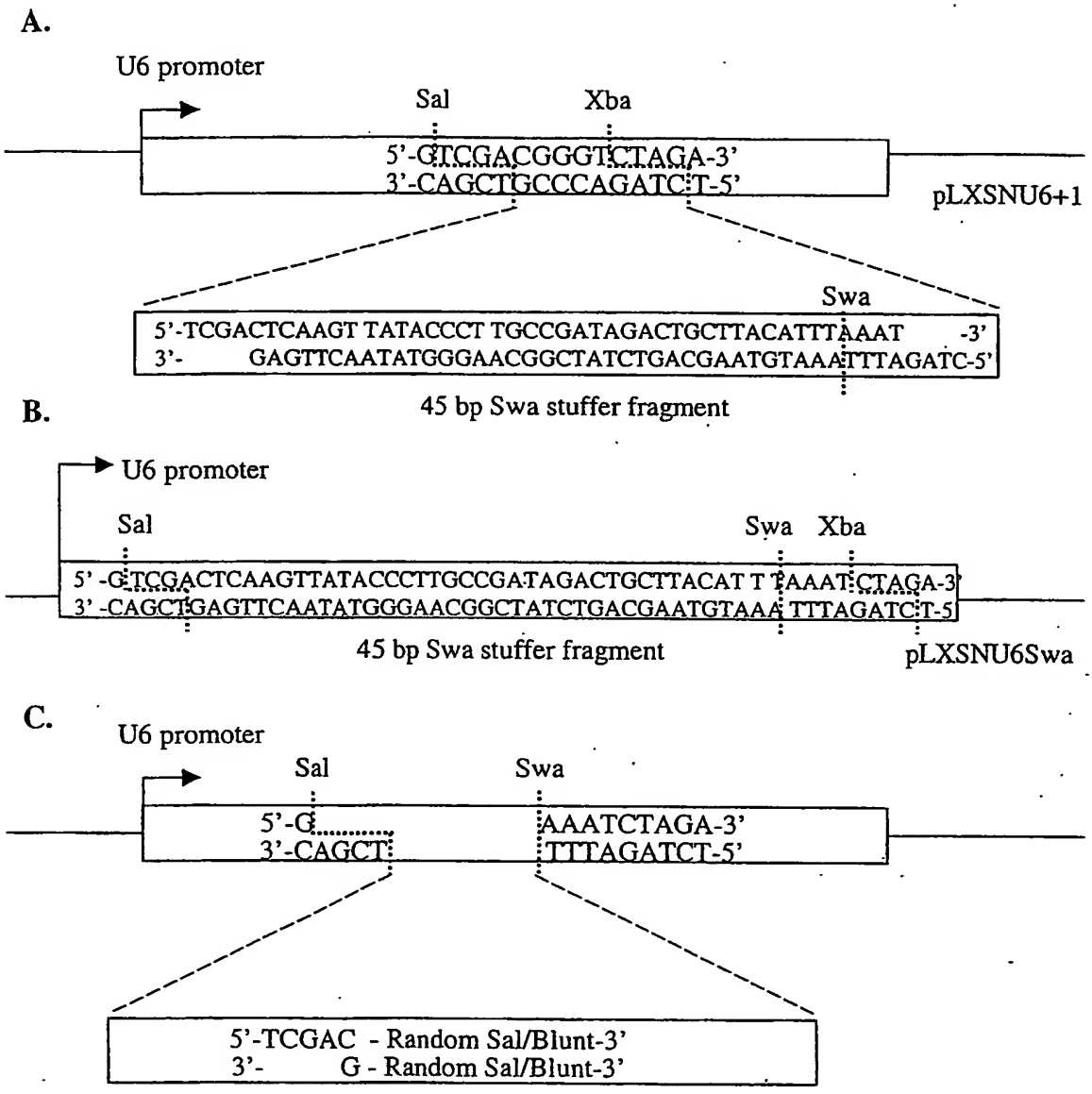


Figure 5. Enzymatic generation of DNA insert encoding complementary sense and antisense RNAs specific for p53.

p53 siRNA Template (63 mer)

5'-*CGGTGATTCCGTCGACC*AAAAAGACTCCAGTGGTAATCTACTTTTT*CTAGAGGTAACAGGCGC*-3'

primer (17 mer) 5'-GCGCCTGTTACCTCTAG-3'

1/ Anneal Primer to p53siRNA

5'-*CGGTGATTCCGTCGACC*AAAAAGACTCCAGTGGTAATCTACTTTTT*CTAGAGGTAACAGGCGC*-3'
3'-GATCTCCATTGTCCGCG-5'

2/ Second Strand Synthesis

5'-*CGGTGATTCCGTCGACC*AAAAAGACTCCAGTGGTAATCTACTTTTT*CTAGAGGTAACAGGCGC*-3'
3'-*GCCACTAAGGGAGCTC*GTTTT TCTGAGGTCACCATTAGATGAAAAA*GATCTCCATTGTCCGCG*-5'

3/ SalI and XbaI Digestion

*TCGACC*AAAAAGACTCCAGTGGTAATCTACTTTTT
GGTTT TTCTGAGGTCACCATTAGATGAAAAA*GATC*

4/ Dephosphorylate and Purify

Figure 6. Reduction in dEGFP-mediated cell fluorescence in cells transiently infected with a retroviral expression vector encoding EGFP siRNA.

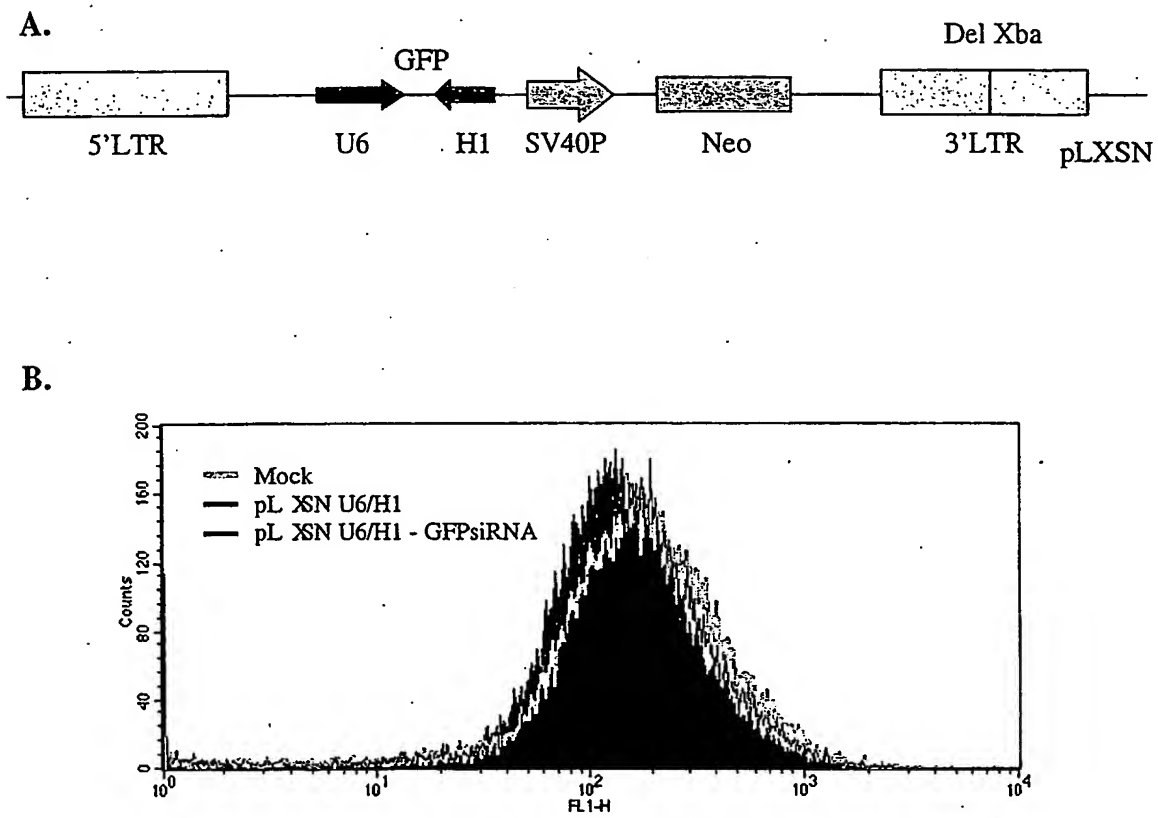


Figure 7. Reduction in p53 protein levels in HCT116 colon carcinoma cells infected with a retroviral expression vector encoding p53 siRNA.

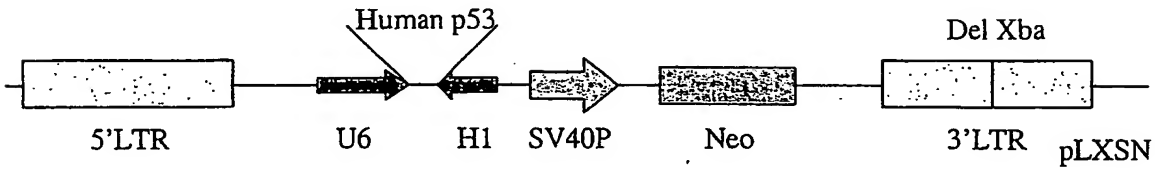


Figure 8. Construction of a genome-wide siRNA retroviral expression library.

A.

19N siRNA Template (63 mer)

5'-*CGGTGATTCCCTCGAG*CAAAAANNNNNNNNNNNNNNNNNNNTTTTT*CTAGAGGTAACAGGCGC*-3'

19N primer (17 mer) 5'-GCGCCTGTTACCTCTAG-3'

1/ Anneal Primer to 19NsiRNA

5'-*CGGTGATTCCCTCGAG*CAAAAANNNNNNNNNNNNNNNNNNNTTTTT*CTAGAGGTAACAGGCGC*-3'
3'-GATCTCCATTGTCCGCG-5'

2/ Second Strand Synthesis

5'-*CGGTGATTCCCTCGAG*CAAAAANNNNNNNNNNNNNNNNNNNTTTTT*CTAGAGGTAACAGGCGC*-3'
3'-*GCCACTAAGGGAGCTC*GTTTT TNNNNNNNNNNNNNNNNNNNAAAAA*GATCTCCATTGTCCGCG*-5'

3/ XhoI and XbaI Digestion

*TCGAC*CAAAAANNNNNNNNNNNNNNNNNNNTTTTT
GGTTT TTNNNNNNNNNNNNNNNNNNNAAAAA*GATC*

4/ Dephosphorylate and Purify

B.

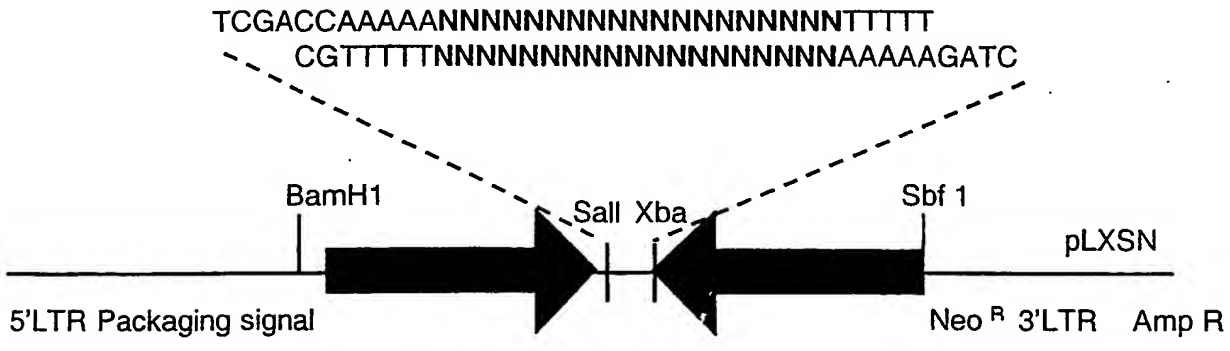


Figure 8. Construction of a genome-wide siRNA retroviral expression library.

C.

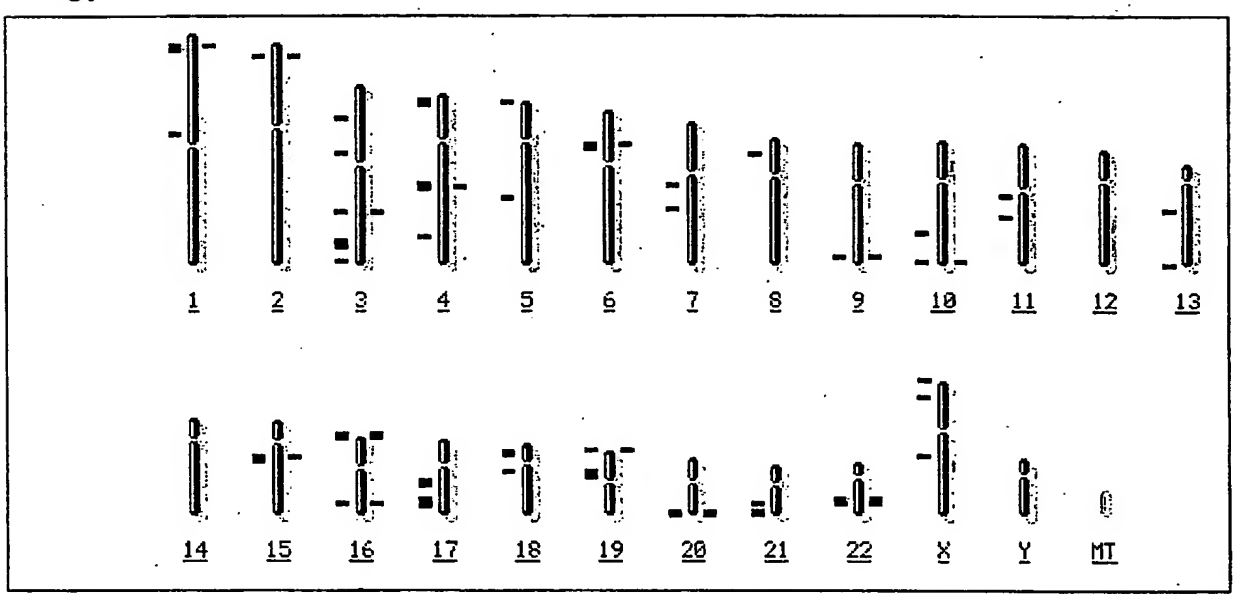
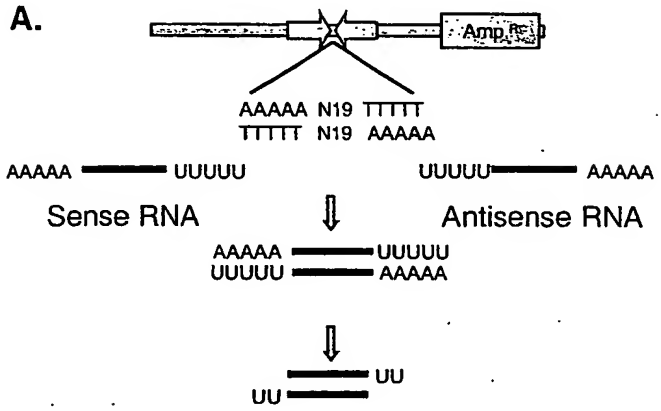


Figure 9. Strategy for generating intracellular siRNAs and effect of the expressed siRNAs on transgene expression.



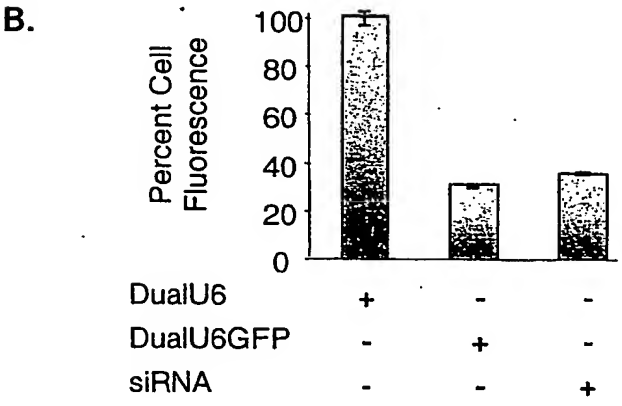


Figure 10. Suppression of dEGFP transgene expression using a stably integrated convergent transcription vector.

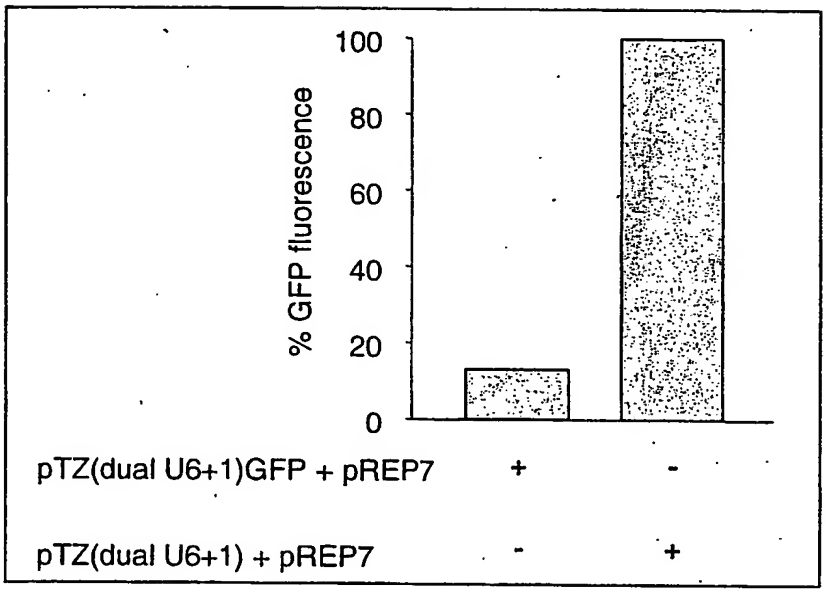


Figure 11. Suppression of target gene expression by the DualU6GFP vector requires the co-expression of both sense and antisense RNAs.

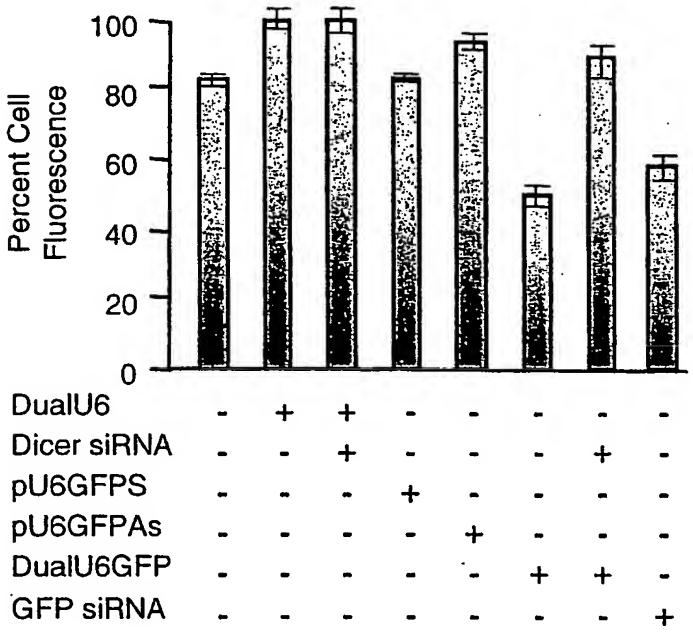


Figure 12. The DualU6GFP expression vector reduces dEGFP target gene expression in a Dicer-dependent manner.

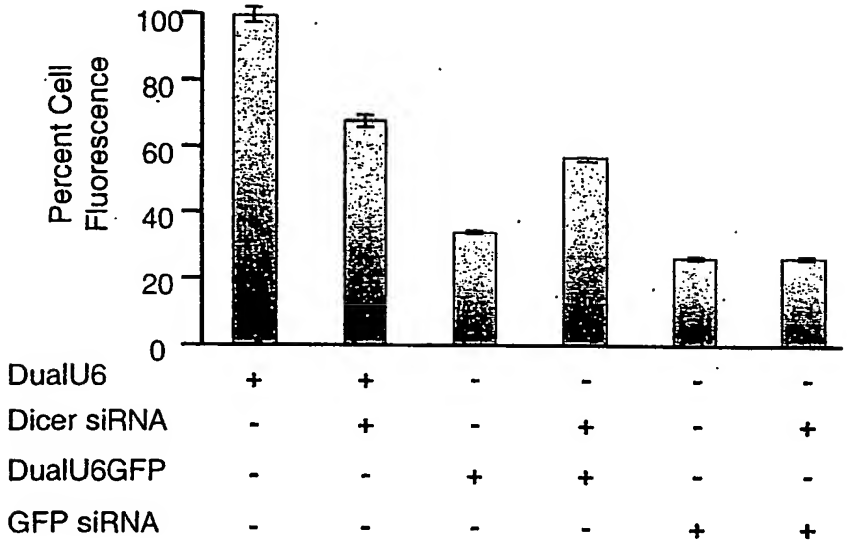


Figure 13. 5-FU-induced apoptosis in HCT116 cells containing pLXSNU6/H1p53.

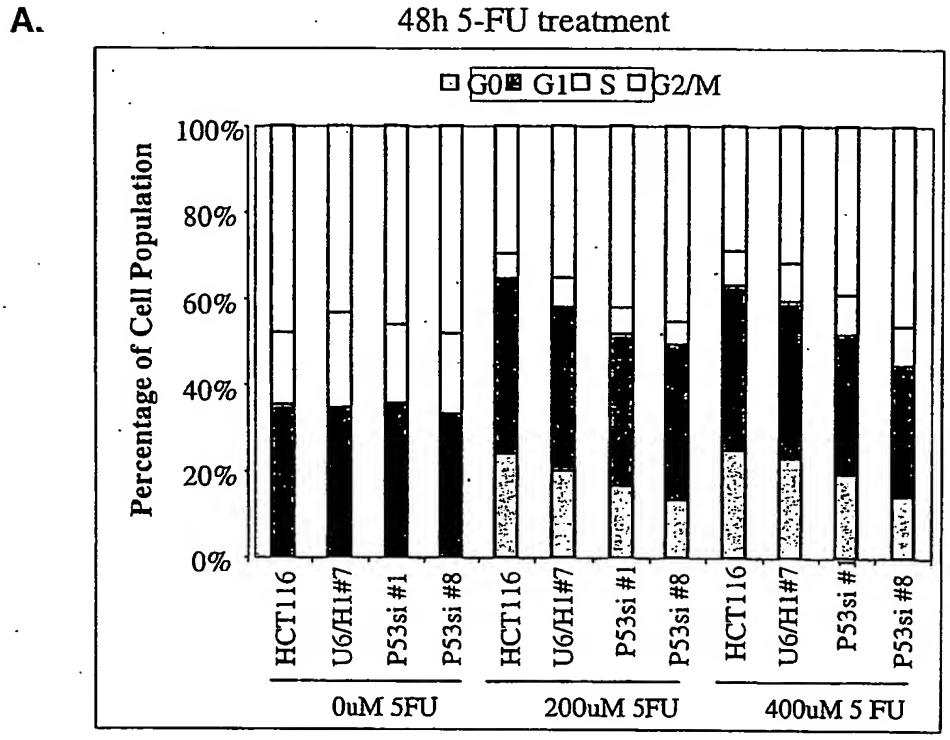


Figure 13. 5-FU-induced apoptosis in HCT116 cells containing pLXSNU6/H1p53.

B.

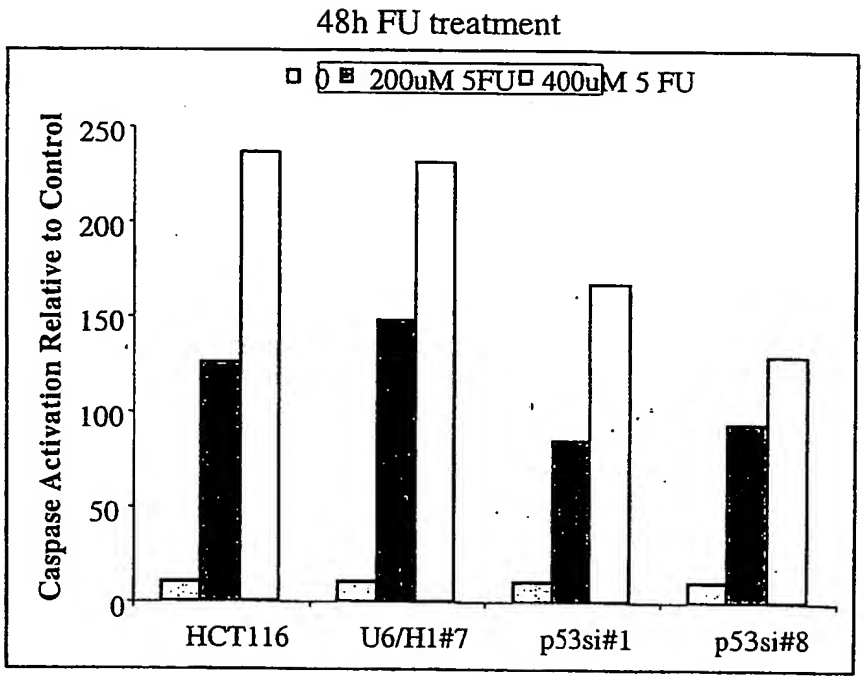


Figure 13. 5-FU-induced apoptosis in HCT116 cells containing pLXSNU6/H1p53.

C.

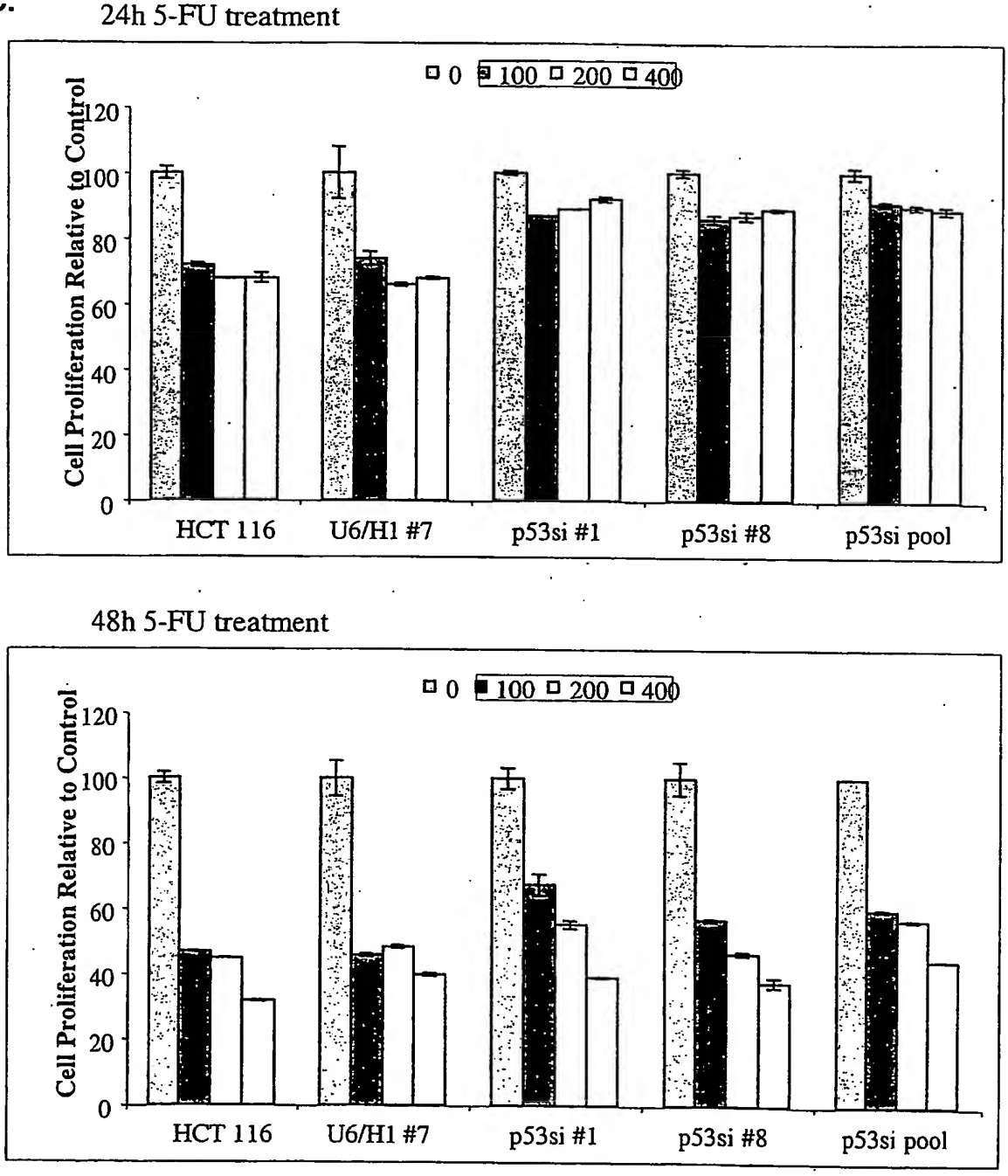


Figure 14. Overview of the 5-FU genetic selection of spiked siRNA expression libraries.

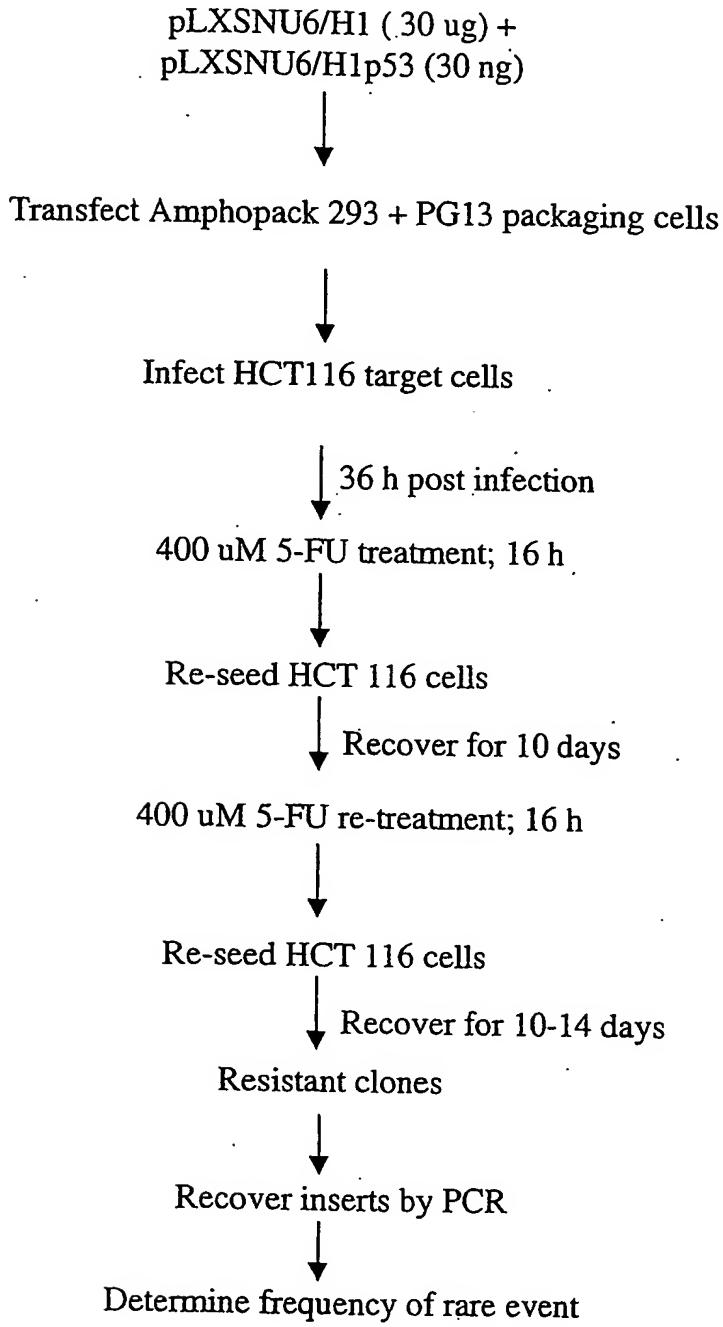


Figure 15. Retroviral expression vectors for genome-wide RNAi libraries.

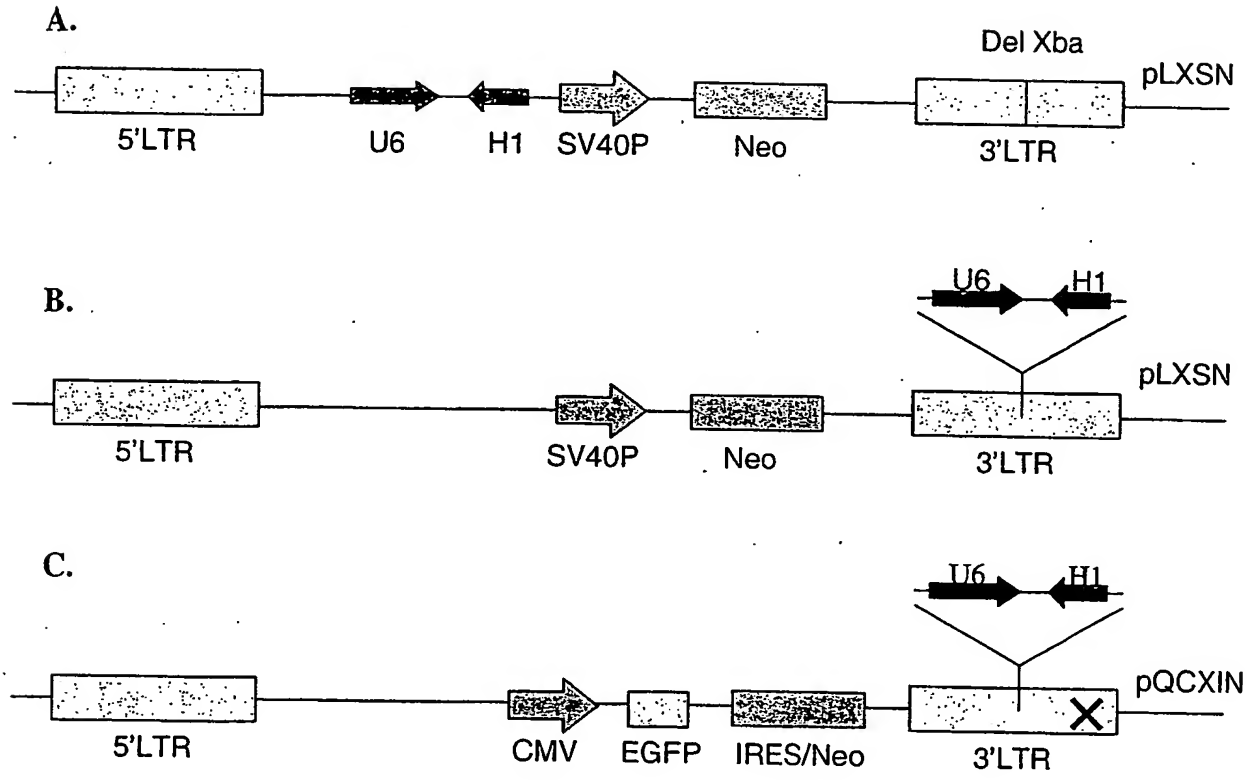


Figure 16. Method for constructing genome-specific shRNA and siRNA libraries

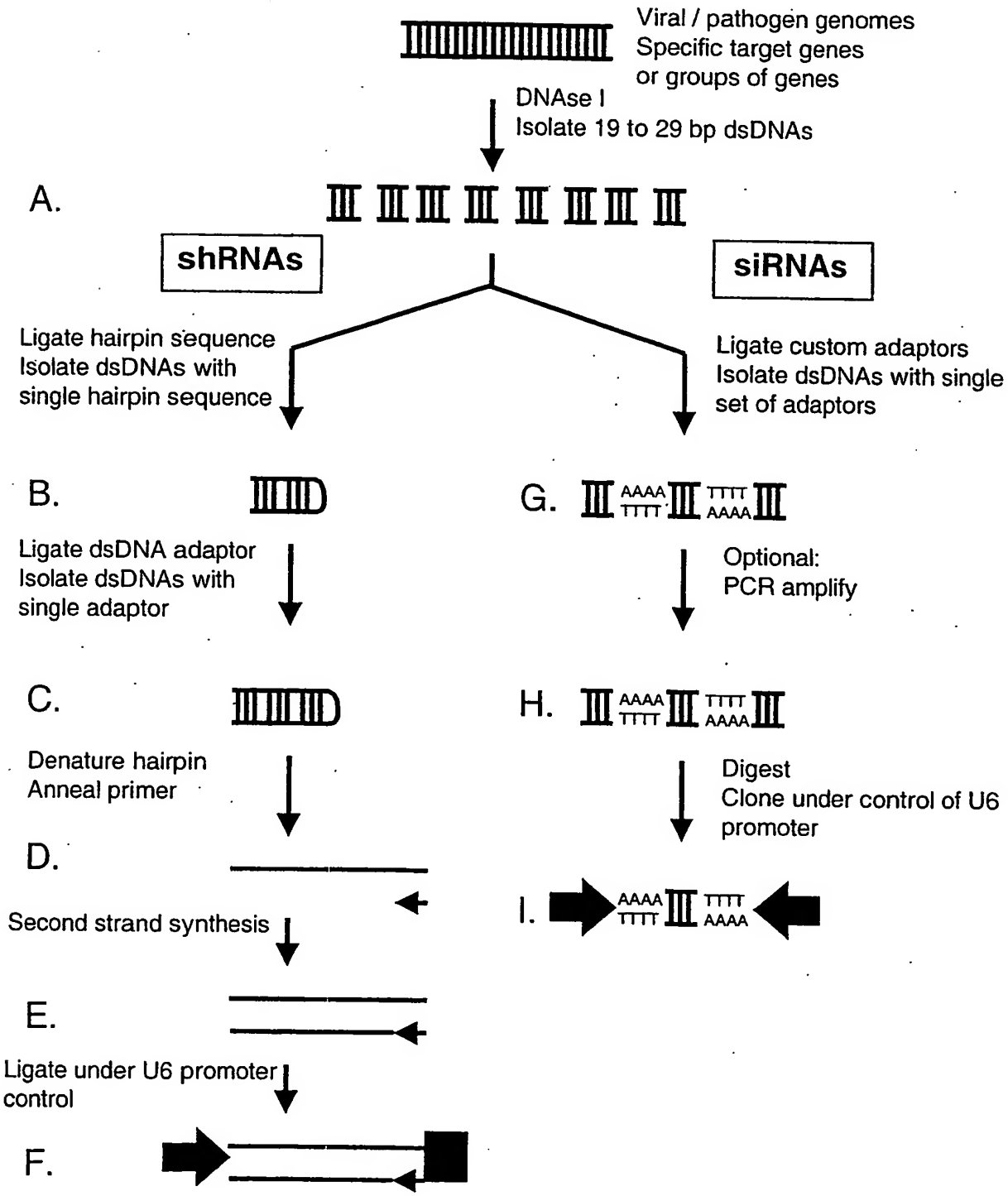


Figure 17. Schematic overview of the method for constructing shRNA and siRNA libraries specific for an expressed RNA population

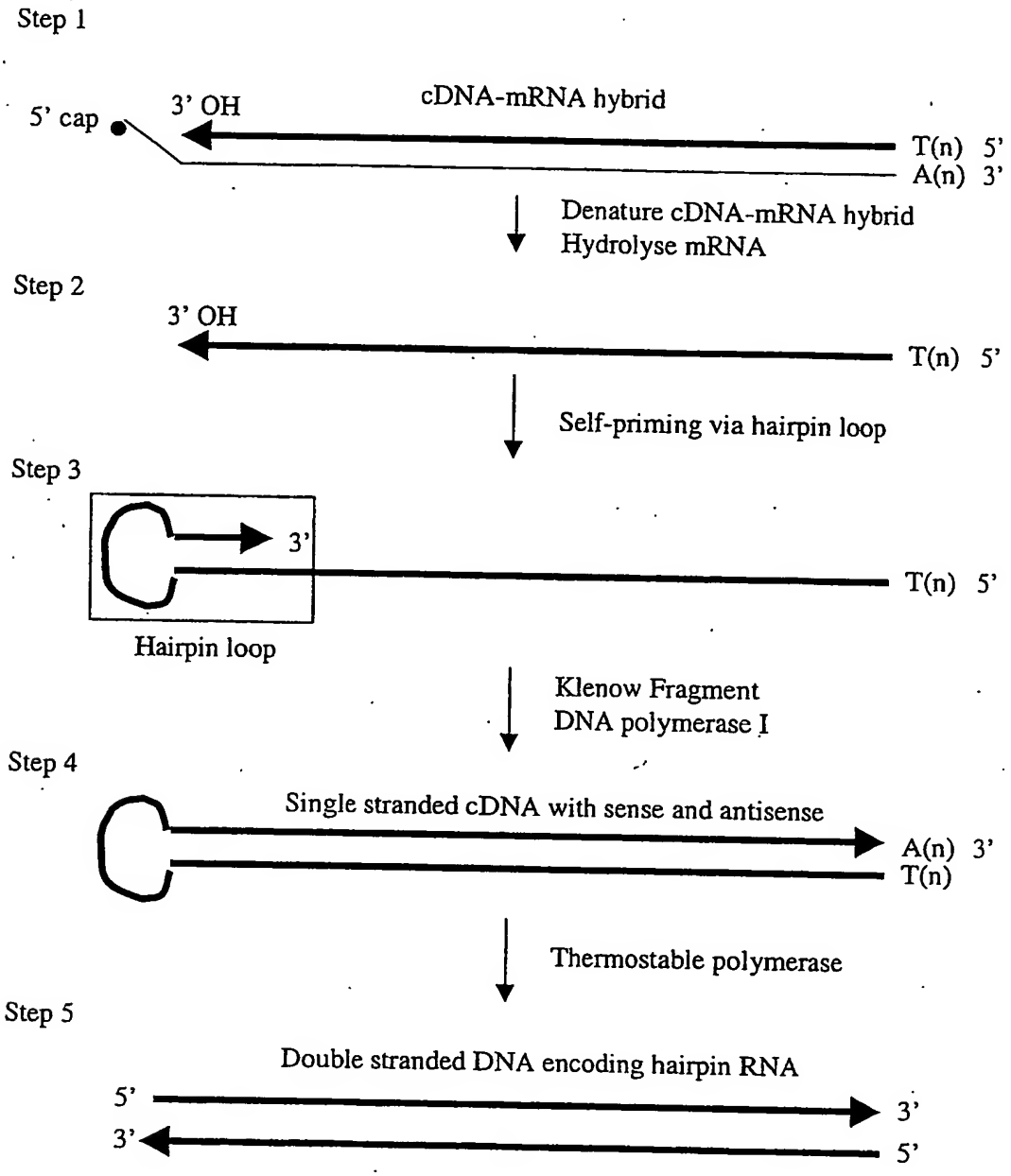
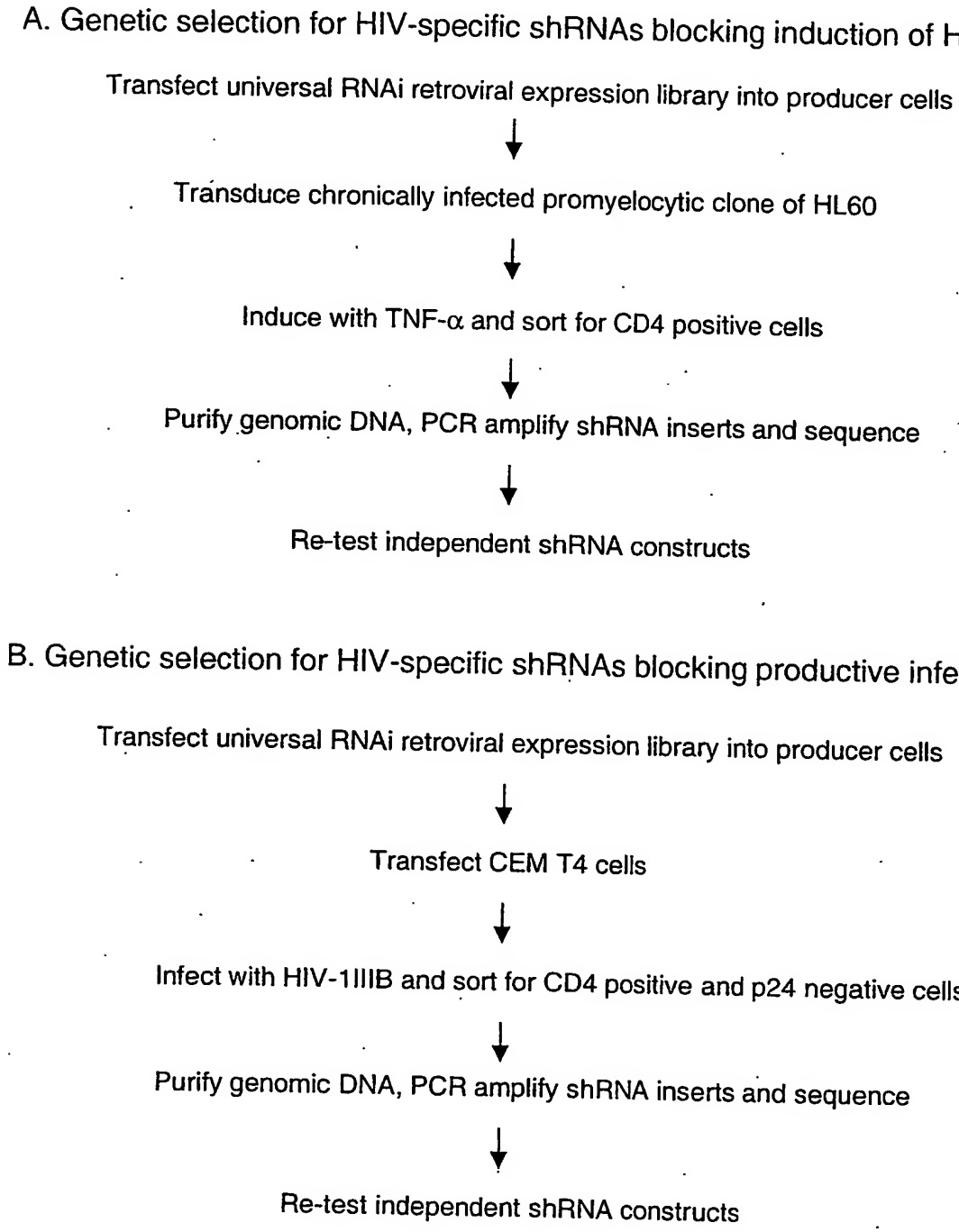


Figure 18. Identification of HIV-specific shRNA or siRNAs using genetic selections



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